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Involvement of the medial amygdaloid nucleus in restraint stress-induced pressor responses in rats

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Abstract

Restraint stress increased the number of neurons with Fos immunoreactivity in the medial amygdaloid nucleus in rats and caused an increase in blood pressure. The stress-induced pressor response was inhibited by muscimol (80 pmol), a neuroinhibitory compound, injected bilaterally into the medial amygdaloid area, whereas muscimol (8 pmol) similarly injected had only a tendency of inhibition of the pressor response. These data suggest that the medial amygdaloid nucleus is involved in mediation of the restraint stress-induced pressor response.

Keywords: Medial amygdala; Restraint stress; Pressor response; Muscimol; Fos immunoreactivity; Rat

Stress causes stimulation of the cardiovascular system and subsequent hypertension [6,10]. However, little is known of the exact central nervous system mechanisms underlying the stress response.

Cardiovascular responses to arousal are thought to be mediated at least partly through limbic forebrain nuclei located in the amygdala. Chemical stimulation of the amygdala causes pressor responses [2,8,9,11,17,20]. Acute stress evokes expression of c-fos, a proto-oncogene thought to be a marker of neuronal activity, in the amygdala [1,3–5]. Folkow et al. [7] demonstrated that increases in mean blood pressure induced by the strong form of environmental stress were lower in amygdala-lesioned rats than in sham-operated control rats. In the present study, we investigated in which parts of the amygdala restraint stress evokes expression of Fos immunoreactivity, and whether the Fos immunoreactivity-expressed part in the amygdala is involved in mediation of restraint stress-induced pressor response in rats.

Experiments were performed in male rats (320–340 g). All rats were housed individually in a climate-controlled room. All procedures were carried out in accordance with the guidelines outlined by the Institutional Animal Care and Use Committee of the Showa Pharmaceutical University. All efforts were made to minimize animal suffering.

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.). For blood pressure recording, a polyethylene cannula was inserted into the abdominal aorta [14], and for amygdala microinjection experiments, guide cannulae (26-gauge stainless-steel tubing) were bilaterally lowered to positions 1.0 mm dorsal to injection sites at the medial amygdaloid area (2.8 mm caudal and 3.4 mm lateral to the bregma, and 8.6 mm below the cerebral surface).

Three days after surgery, experiments were started. The arterial catheter was connected to a pressure transducer. After a stabilization period of more than 60 min, each obturator was removed from the guide cannula and replaced with an inner cannula (32-gauge stainless tubing) filled with the agent to be administered. The inner cannula was connected to 5 μl Hamilton syringe and microinjector (IM-1; Narishige, Tokyo, Japan). Drugs were injected into free-moving rats at a dose of 100 nl. Each rat received either one dose of muscimol or vehicle solution (phosphate-buffered saline, pH 7.4).

Restraint stress was initiated by putting the rat in a small cylindrical restraining device (diameter 6.5 cm, length 15 cm) (Fisher Scientific, Centrap Cage) that held each rat in a normal standing position [15]. The rat had a 60 min period of

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stress exposure. Injection of a dose or vehicle was performed 5 min before the start of the stress exposure. Eight animals were used in each group. All testing was performed between 10:00 and 14:00 h in order to minimize circadian variation. At the end of each experiment, these animals were deeply anesthetized with pentobarbital and the injection site was marked by injecting concentrated solution of Pontamine sky blue. The brain was fixed and removed, and frozen sections were cut for identification of the injection site.

For Fos experiments, six unoperated intact rats were used, three for the stress experiments and three for the control experiments. Immunocytochemical staining for Fos was performed using Fos antiserum (Calbiochem, MA) as described elsewhere [12]. Staining was performed using VectaStain Elite Kits (Vector Laboratories, Burlingame, CA). The drug used was muscimol (Sigma, St. Louis, MO). The results are expressed as mean ± SEM. All results were analyzed by either Student's *t*-test or one-way analysis of variance combined with Dunnett's test for post-hoc analysis for intergroup comparison. Differences were considered significant at $P < 0.05$.

Unoperated intact rats were used for Fos experiments. Following 60 min of restraint stress, a lot of nuclei with Fos immunoreactivity were detected in the medial amygdaloid nucleus (MeA) (Fig. 1). Six sections were counted through the MeA in each experiment. The numbers of nuclei with Fos immunoreactivity counted at the levels of bregma −2.8 mm and −3.4 mm in the MeA were 84 ± 6 ($n = 3$) and 68 ± 5 ($n = 3$), respectively. In other areas of the amygdala, no or only scatter Fos immunoreactivity was found. In control animals similarly handled but left unstressed for 60 min, no Fos immunoreactivity was detected in the MeA ($n = 3$, data not shown).

Restraint stress caused increases in blood pressure in rats given vehicle in both sides of the MeA (Fig. 2A,B). When muscimol (80 pmol), a $GABA_A$ receptor agonist, was injected bilaterally into the MeA 5 min before restraint, the

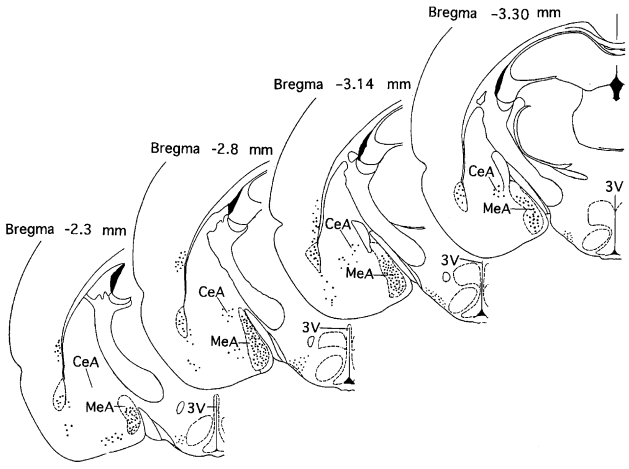

Fig. 1. Distribution of Fos immunoreactive neurons in the amygdala of a rat subjected to 60 min restraint. CeA, central amygdaloid nucleus; MeA, medial amygdaloid nucleus; 3V, third ventricle.

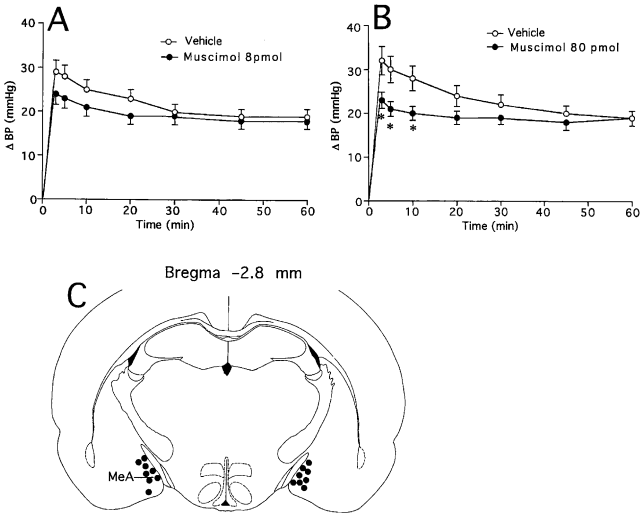

Fig. 2. (A,B) Effect of bilateral microinjection of muscimol, 8 (A) and 80 (B) pmol, into the MeA on blood pressure responses induced by restraint stress in rats. Changes in mean blood pressure (ΔBP) induced by 60 min restraint stress are shown. Muscimol or vehicle was given 5 min before the period of the stress. The basal mean blood pressure in the vehicle groups, the muscimol 8 pmol group, and the muscimol 80 pmol group was 97 ± 0.9 mmHg ($n = 16$), 98 ± 1.2 mmHg ($n = 8$), and 96 ± 1.4 mmHg ($n = 8$), respectively. Values are means ± SEM from eight animals. $^*P < 0.05$, compared with respective vehicle. (C) Schematic diagram of the transverse section of rat forebrain, showing the sites where MeA microinjections of muscimol (80 pmol) inhibited stress-induced pressor responses.

pressor response induced by restraint stress was inhibited (Fig. 2B). When muscimol (8 pmol) was injected similarly, a tendency of inhibition of the pressor response was found (Fig. 2A). Injection of vehicle or muscimol (8 and 80 pmol) did not affect basal blood pressure. Postmortem histological examination confirmed that MeA injection sites were located in areas containing the medial amygdala (Fig. 2C).

In the present study, restraint stress increased the number of neurons with Fos immunoreactivity in the MeA, suggesting that restraint stress causes an activation of neurons in the MeA. Restraint stress caused pressor responses and the stress-induced pressor response was inhibited by the neuroinhibitory compound, muscimol, injected bilaterally into the MeA. These findings suggest that the MeA is involved in mediation of pressor responses induced by restraint stress.

The results of the present study confirmed the findings of Chen and Herbert [4] and Cullinan et al. [5] showing that restraint stress induces c-fos in the MeA. Although we found scatter Fos immunoreactivity in the central nucleus of the amygdala in rats after restraint stress, the central nucleus of the amygdala is also thought to be important for responses to stress. Makino et al. [16] have demonstrated that psychological stress increases corticotropin-releasing hormone mRNA and content in the central nucleus of the amygdala in rats. Iwata et al. [11] have demonstrated that cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus resemble conditioned emotional responses in rats. In addition, it has been

demonstrated that central amygdaloid lesions attenuate cardiovascular responses to intermittent foot shock in rats [19]. More studies will be needed to clarify the roles of the central amygdala and the medial amygdala in mediating cardiovascular responses to stress.

Previously, we demonstrated that angiotensin AT1 receptor blockade in the anterior hypothalamic area inhibited the pressor response induced by restraint stress in rats [15]. Furthermore, activation of angiotensin AT1 receptors in the anterior hypothalamic area produced pressor responses via cholinergic inputs to the rostral ventrolateral medulla, relaying in the paraventricular hypothalamic nucleus [13]. A recent study using combined anterograde/retrograde tract tracing has demonstrated that medial amygdala neurons connect with hypothalamic paraventricular nucleus neurons, relaying in the anterior hypothalamic area [18]. Thus, it can be speculated that the medial amygdala play a role in anterior hypothalamic neuron responses involved in restraint stress-induced pressor responses.

In the present study, the difference between the vehicle- and muscimol-injected rats disappeared by 60 min, while muscimol is a very long-lasting neuroinhibitory compound. Thus, it can be speculated that there may be two components to the pressor response – an immediate effect which gradually disappears after 60 min, and a more stable component which continues as long as the stress persists. It is probable that muscimol inhibited only the former component.

In summary, restraint stress increased the number of neurons with Fos immunoreactivity in the MeA, and muscimol injected into the MeA inhibited the stress-induced pressor response. These findings provide the first evidence that the MeA is involved in mediation of the restraint stress-induced pressor response.

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